

## SUBCLINICAL SALMONELLA INFECTION IN DANISH FINISHING PIG HERDS: ASSOCIATION BETWEEN SEROLOGICAL AND BACTERIOLOGICAL TESTING

Stege H.<sup>ab</sup>, Carstensen B.<sup>a</sup>, Christensen J.<sup>a</sup>, Feld N. C.<sup>b</sup>,  
Baggesen D. L.<sup>a</sup>, Nielsen J. P.<sup>a</sup>

As part of the salmonella surveillance programme in Danish slaughter pig herds (Mousing et al. 1997), the occurrence of subclinical salmonella infection in pigs is monitored serologically at slaughter by examination of meat juice samples, using a mix-ELISA (Nielsen et al. 1997).

Subsequently blood samples and pen (faecal) samples are used as diagnostic tests in infected herds. The association between the serological and the bacteriological testing therefore needs assessment. The objective of the present study was to assess the association between serological and bacteriological testing.

### MATERIALS AND METHODS

The study comprised 96 finishing herds randomly selected among herds that were using the Integrated Farm Management System, and 39 herds with a high sero-prevalence in the surveillance programme. From each herd, 10 pens were examined by: (1) a pooled pen sample (5x5 g faeces), (2) a feed sample (50 g feed), and (3) 5 blood samples. All samples were forwarded to the Danish Veterinary Laboratory and examined by culturing or in the mix-ELISA (Nielsen et al. 1995). The data included the results of bacteriological testing of pen and feed samples, stated as salmonella serotype or negative, and the result of the serological testing, stated as OD% (optical density).

In the analyses, the results of the bacteriological testing of feed and pen samples and salmonella serotypes were categorized as either '*S. Typhimurium*' or 'non- *S. Typhimurium*'.

In this study the following definitions were applied: A herd was defined bacteriologically positive if *S. Typhimurium* was isolated from one or more pen(s). A pig was considered sero-positive when OD% > 10 (Nielsen et al. 1995). For each herd, the prevalence of sero-positive pigs was calculated.

To test the association between the bacteriological findings and the serological response at the herd level, linear regression (SAS, ver 6.11) was applied. The dependent variable was log(proportion of individual OD% > 10). The independent variables, included in the model was: Proportion *S. Typhimurium* positive feed samples, proportion *S. Typhimurium* positive pen samples, proportion non-*S. Typhimurium* positive pen samples and proportion non-*S. Typhimurium* positive feed samples. The significance level  $\alpha = 0.1$  was used, and the selection method was backwards.

In order to study the relation between serological and bacteriological testing, predictive values of herd diagnoses based on prevalence of sero-positive pigs were calculated. The predictive value of a positive serological herd diagnosis ( $P\{B+|S+\}$ ) is the probability of testing a herd bacteriologically positive, provided the herd tested serologically positive. The predictive value of a negative serological herd diagnosis ( $P\{B-|S-\}$ ) is the probability of testing a herd

---

<sup>a</sup> Danish Veterinary Laboratory, Bülowsvej 27, DK-1790 Copenhagen V, Denmark.

<sup>b</sup> Royal Veterinary and Agricultural University, Bülowsvej 13, 1870 Frederiksberg C, Denmark

bacteriologically negative, provided the herd tested serologically negative.

The predictive values were plotted against the herd prevalence of sero-positive pigs used for defining a sero-positive herd. This was done for pigs considered sero-positive at  $OD\% > 10$  and  $OD\% > 40$ . A herd was considered bacteriologically positive if *S. Typhimurium* was isolated from one or more pen sample(s).

## RESULTS

No positive correlation between proportion of positive samples was found when comparing pen and feed samples (Figure 1a). Generally, the salmonella serotypes isolated from feed samples were non-*S. Typhimurium* while *S. Typhimurium* was predominating in the pen samples. No positive correlation between proportion of positive samples was found when comparing feed samples and the herd sero-prevalence (Figure 1b).

The resulting model after linear regression and backwards elimination of proportion non-*S. Typhimurium* positive feed samples, proportion *S. Typhimurium* positive feed samples, and proportion non-*S. Typhimurium* positive pen samples, only included the proportion of *S. Typhimurium* positive pen samples as significant at the 0.1 level. (t- statistic = 2.59 on 133 d.f.) (Figure 1c).

The association between serological and bacteriological testing appears in figure 2 (96 randomly selected herds) and figure 3 (39 high sero-prevalence herds).

When only including the 96 randomly selected herds, the positive predictive value of a serological herd diagnosis ( $P\{B+|S+\}$ ), when considering pigs sero-positive at  $OD\% > 10$ , ranged from 14% at herd sero-prevalence 0% to 100% at herd sero-prevalence 34%. When considering pigs sero-positive at  $OD\% > 40$ , the corresponding positive predictive values ranged from 14% at herd sero-prevalence 0% to 100% at herd sero-prevalence 15%.

The negative predictive value of a serological herd diagnosis ( $P\{B-|S-\}$ ), when considering pigs sero-positive at  $OD\% > 10$ , ranged from 100% at herd sero-prevalence 0% to 86% at herd sero-prevalence 78%. When considering pigs sero-positive at  $OD\% > 40$ , the corresponding predictive value ranged from 91% at herd sero-prevalence 0% to 86% at herd sero-prevalence 34% (Figure 2).

When only including the 39 high sero-prevalence herds, the positive predictive value of a serological herd diagnosis ( $P\{B+|S+\}$ ), when considering pigs sero-positive at  $OD\% > 10$ , ranged from 77% at herd sero-prevalence 0% to 100% at herd sero-prevalence 70%. When considering pigs sero-positive at  $OD\% > 40$ , the corresponding positive predictive values ranged from 77% at herd sero-prevalence 0% to 100% at herd sero-prevalence 32%.

The negative predictive value of a serological herd diagnosis ( $P\{B-|S-\}$ ), when considering pigs sero-positive at  $OD\% > 10$ , ranged from 67% at herd sero-prevalence 11% to 23% at herd sero-prevalence 95%. When considering pigs sero-positive at  $OD\% > 40$ , the corresponding predictive value ranged from 75% at herd sero-prevalence 5% to 23% at herd sero-prevalence 70% (Figure 3).

## DISCUSSION

According to the results of this study, a relationship between proportion of *S. Typhimurium* positive pen samples and sero-prevalence was observed at the herd level. No relation between positive feed samples or 'non-*S. Typhimurium*' positive pen samples and sero-prevalence was

observed at the herd level.

The association of serological and bacteriological testing depends on the criteria used for defining a sero-positive herd. When requiring relatively high prevalence of sero-positive pigs in order to define a sero-positive herd, the probability of having a positive bacteriological herd diagnosis in a sero-positive herd increased while the probability of having a negative bacteriological herd diagnosis decreased. The lower negative predictive values calculated for the 39 high sero-prevalence herds compared to the 96 randomly selected herds, are mainly caused by the fact that the high sero-prevalence herds have higher infection levels. This also explains the differences in the positive predictive values that were found higher for herd sero-prevalences below 15%, but lower above this point, since the 39 high sero-prevalence herds (meat juice) include only a very small fraction of herds with low sero-prevalence (blood).

Looking at the 96 randomly selected herds it was found that when using a definition of test positive herds at a herd sero-prevalence of 35% and considering pigs sero-positive at OD% > 10, a high probability of obtaining a bacteriological positive herd diagnosis in sero-positive herds and an almost equally high probability of obtaining a bacteriological negative diagnosis in sero-negative herds was demonstrated (P = 100% and 93% respectively) Figure 2.

Looking at the 39 high sero-prevalence herds and using a definition of a test positive herd at a sero-prevalence of 50% and considering pigs sero-positive at OD% > 40, a very high probability of obtaining a bacteriological positive herd diagnosis in sero-positive herds and a low probability of obtaining a bacteriological negative diagnosis in sero-negative herds was demonstrated (P = 100% and 26%, respectively) Figure 3.

In the Danish *Salmonella enterica* surveillance and control programme, herds delivering between 100 and 5000 pigs for slaughter annually are considered highly infected (level 3) if the prevalence of sero-positive meat juice samples (OD% > 40) is > 50% (Mousing et al. 1997). Practical experience from the programme has demonstrated a lower probability (52/69=75%) of isolating *S. Typhimurium* in level 3 herds (Christensen et al. 1997) than demonstrated in this study. Possible explanations of this difference may be that in this study serum was used instead of meat juice, the number of pen samples examined were 10, and the samples were collected simultaneously in this study.

## REFERENCES

- Christensen, J., Hillersborg, M., Baggesen, D.L., 1997. Detection of *Salmonella enterica* in subclinically infected herds. Second International Symposium on Epidemiology and Control of Salmonella in Pork. August 20-22, 1997, Copenhagen, Denmark.
- Mousing, J., Thode Jensen, P., Halgaard, C., Bager, F., Feld, N., C., Nielsen, B., Nielsen, J.P., Bech-Nielsen, S., 1997. Nation-wide *Salmonella enterica* surveillance and control in Danish slaughter swine herds. Prev. Vet. Med., in press.
- Nielsen, B., Baggesen, D.L., Bager, F., Haugegaard, J., Lind, P., 1995. The serological response to *Salmonella* serovars Typhimurium and Infantis in experimentally infected pigs. The time course followed with an indirect anti-LPS ELISA and bacteriological examinations. Veterinary Microbiology 47: 205-218.
- Nielsen, B., Ekeröth, L., Bager, F., Lind, P., 1996. Use of muscle juice as a source of antibodies for large scale serological surveillance of *Salmonella* infection in slaughter pig herds. Submitted

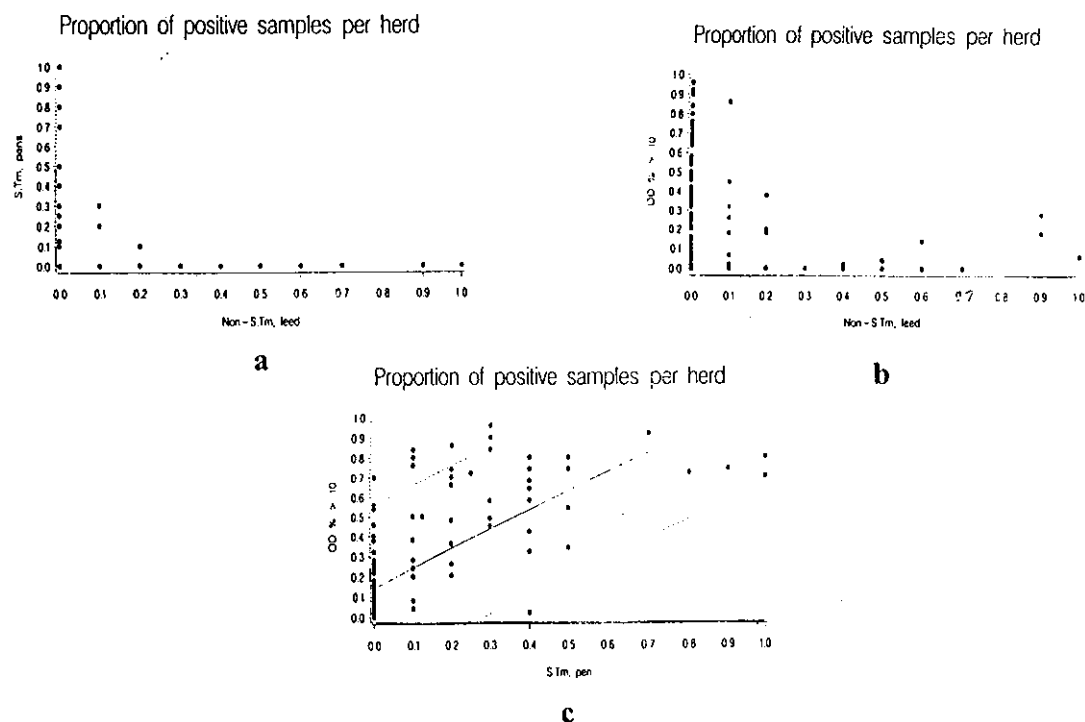
to J. Vet. Diag. Invest.

SAS Institute Inc., SAS/STAT® User's Guide, Version 6, *Fourth Edition, Volume 1*, Cary, NC: SAS Institute Inc., 1989. 943 pp.

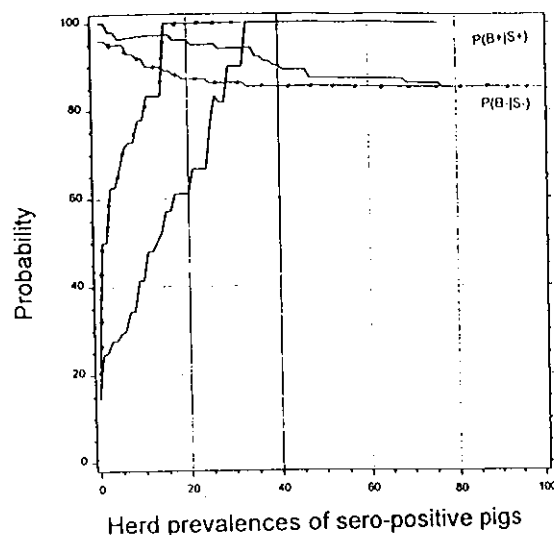
SAS Institute Inc., SAS® *Language: Reference*, Version 6, *First Edition*, Cary, NC: SAS Institute Inc., 1990. 1042 pp.

## SUMMARY

The salmonella surveillance programme in Danish slaughter pig herds includes serological monitoring of subclinical salmonella infections. As part of the interventions in infected herds a bacteriological follow-up is performed. The agreement of serological and bacteriological testing therefore needs assessment. The objective of the present study was to assess the association between serological and bacteriological testing at the herd level. 135 herds participated in the study and were examined by blood, feed and pen samples from 10 pens per herd. Generally the salmonella serotypes isolated from feed samples were non-*S. Typhimurium*, while *S. Typhimurium* was dominating in the pen samples. A positive linear relation between proportion of *S. Typhimurium* positive pen samples and sero-prevalence was observed at herd level. No relation between positive feed samples or 'non-*S. Typhimurium*' positive pen samples and sero-prevalence was observed at herd level. The probability of testing a herd bacteriologically positive, provided that the herd was serologically positive, depended on the criteria for definition of sero-positive pigs and herds.

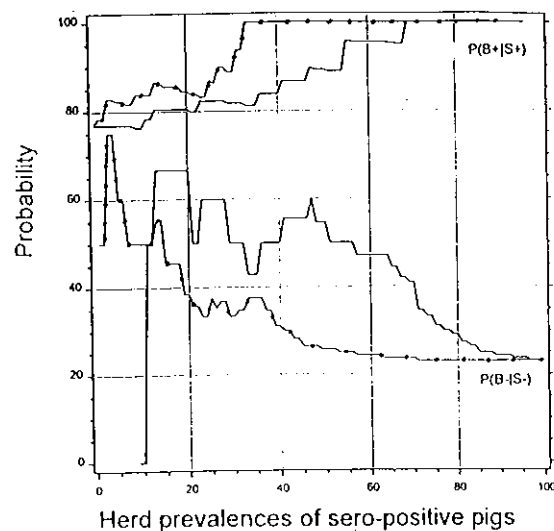


**Figure 1. Bacteriological and serological findings in feed (n=10), pen (n=10) and serum (n=50) samples from 135 Danish finishing herds. Tm = *S. Typhimurium*. a) Proportion of positive feed and pen samples. b) Proportion of positive feed pen samples and herd prevalence of sero-positive pigs (OD% > 10). c) Proportion of positive pen samples and herd prevalence of sero-positive pigs (OD% > 10).**



**Figure 2. Agreement of serological and bacteriological herd diagnoses. 96 randomly selected Danish finishing herds were defined as bacteriologically positive if *S. Typhimurium* was isolated from one or more pen sample(s). Pigs were defined as sero-positive at OD% > 10 (—) and OD% > 40 (----).**

**B+ = Bacteriologically positive. B- = Bacteriologically negative. S+ = Serologically positive. S- = Serologically negative.**



**Figure 3. Agreement of serological and bacteriological herd diagnoses. 39 high sero-prevalence herds were defined as bacteriologically positive if *S. Typhimurium* was isolated from one or more pen sample(s). Pigs were defined as sero-positive at OD% > 10 (—) and OD% > 40 (----).**

**B+ = Bacteriologically positive. B- = Bacteriologically negative. S+ = Serologically positive. S- = Serologically negative.**